Applied Biological Research 25(4): 381-389; (2023)
DOI: 10.5958/0974-4517.2023.00044.7

DEVELOPMENT OF HYDROPHOBIC OIL-RESISTANT PAPER USING LATEX OF *Artocarpus heterophyllus* Lam. AND PRELIMINARY PHYTOCHEMICAL, ANTIOXIDANT AND ANTIBACTERIAL STUDIES OF ITS SEEDS AND LEAVES

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(Received 24 May, 2023; accepted 12 August, 2023)

ABSTRACT

The present study was aimed to develop hydrophobic and oil-resistant paper using *Artocarpus heterophyllus* Lam. latex and to analyse its antioxidant properties, nutritional value, functional groups in seeds and phytochemicals, and antibacterial activity of seeds and leaves. The DPPH assay method (antioxidant activity), FTIR analysis (identification of functional groups) and the disc diffusion method (antibacterial activity) were used. The chloroform is the better solvent for latex extraction than hexane, with hydrophobicity of 50 min and an oil resistance of 1.5 h. Hexane extract had a hydrophobicity of 25 min and an oil resistance of 1 h. The proximate analysis of seeds revealed the presence of carbohydrates as major constituent (69.59%), followed by protein (10.77%), moisture (11.31%), ash (6.08%), and fat (2.25%). The methanol extracts of seeds and leaves contained secondary metabolites like tannins, saponins (in seeds), flavonoids and phenols (in leaves). Chloroform extracts of *A. heterophyllus* seeds showed 8.2 mm zone of inhibition against *Staphylococcus aureus* (MTCC 96) and 8.8 mm against *Escherichia coli* (MTCC 443), while leaves did not show any significant inhibition. The study emphasizes the usage of *A. heterophyllus* to develop various eco-friendly products and to exploit the nutritional and mineral contents and the antioxidant and antibacterial properties.

Keywords: Antibacterial activity, *Artocarpus heterophyllus*, hydrophobic, latex, oil resistant, paper

INTRODUCTION

Jackfruit (*Artocarpus heterophyllus* Lam.), the world's largest tropical tree-borne fruit, belongs to the family Moraceae. It is an evergreen monoecious tree that is presumably native to the Western Ghats rain forests in the Southwest of India (Baliga *et al.,* 2011). The tree normally grows to a height of 10 to 20 m with stem diameter of 30 to 80 cm in tropics. It has a very thin trunk and extensive side branching that typically starts close to the ground, giving the appearance of a highly dense treetop. The bark is 1.25 cm thick, green or black, and has a straight, rough stem. When the tree is wounded, it releases a sticky white latex. The leaves of juvenile plants are leathery, stiff, elliptic to ovate-shaped, large (up to 16 cm long), alternating, whole, simple, glossy and highly lobed. This species has greenish flowers that are monoecious with male and female inflorescences growing in different flower heads. Strong stems emerge from older branches, and the trunk bears female spikes. On younger branches, male spikes are located above female spikes. The fruits are compound or multiple fruits (syncarp) with a thick, rubbery, whitish to yellowish wall and an outer rind made of hexagonal, bluntly conical carpel apices. Its seeds are spherical, light brown to brown, 2-3 cm long, and 1-1.5 cm wide having a thin whitish membrane covering (Menaka *et al.,* 2011).

Jackfruit, a staple food in India, is available in markets from spring to summer under the names kanthal, kathal and kathar. It is widely grown throughout Southeast Asia (Rahaman *et al.,* 1999), including in Sri Lanka, Malaysia, Indonesia, Thailand, Philippines, Bangladesh and India (Baliga *et al.*, 2011). The rich pulp of ripe jackfruit is consumed fresh as a dessert, and added to fruit salads, whereas tender green fruit is used as a vegetable. The seeds are cooked, roasted or fried (Sreeletha *et al.*, 2018). In traditional medicine, jackfruit is also used to cure conditions like asthma, ulcers, wound healing, dermatitis, cough, hypertension, anxiety and constipation (Abdul and Martin, 2015). *A. heterophyllus* reportedly has anti-inflammatory, antibacterial, antioxidant and anti-diabetic properties (Fernado *et al.,* 1991; Ko *et al.,* 2005).

The latex of *A. heterophyllus* contains lipid-derived waxy substances, proteins, secondary metabolites and other minerals. In present study, the latex of *A. heterophyllus* was used to treat the paper in order to improve its properties like hydrophobicity and oil resistance, which are considered important factors for food packaging. Hydrophobicity is the partitioning of non-polar compounds between water and a non-aqueous phase (Andrews, 1997). Hydrophobic paper is essential because the majority of people today use plastic bags as food containers and packaging. The chemicals in plastic make it harmful to use, especially for food. Hydrophobic papers can serve as an alternative to plastics because they can be recycled, are biodegradable, and are created from renewable resources (Maria *et al.*, 2019). Paper is frequently coated with different polyolefin substances to increase hydrophobicity. Due to this coating, the paper loses its ability to biodegrade and is more difficult to recycle. A natural hydrophobic material that can give paper hydrophobicity at a reasonable cost is urgently needed to solve these issues and assure sustainable development (Jyoti *et al.*, 2014).

Oil resistance can be defined as the capacity of a natural rubber product to carry out its intended function when in contact with oil (Peter *et al.,* 1999). It can be considered an important feature of papermaking industry. The physical and mechanical qualities of common paper materials are remarkable; however, they are inadequate for gas and oil resistance. As a result, specific procedures or additives are frequently used to improve their oil resistance qualities. The most popular technique for increasing the oil resistance of papers is to incorporate a fluorine-based oil-resistant chemical into the paper itself (Zhu *et al.*, 2015). The chemicals resistant to perfluoro may transfer into food and raise the risk of cancer (Lau *et al.,* 2004; Cui *et al.*, 2009). The creation of novel, non-toxic and ecologically acceptable oil-resistant agents is emerging as an innovative research topic. The objective of present study was to develop *Artocarpus heterophyllus* latex-coated hydrophobic and oil-resistant paper and to ascertain antioxidant activity, physicochemical characteristics and functional groups of its seeds, along with phytochemical detection and antibacterial action of its seeds and leaves.

MATERIALS AND METHODS

The latex, seeds and leaves of *Artocarpus heterophyllus* were collected from local market in Palakkad. The plant was identified using the Flora of Presidency of Madras (Gamble and Fischer, 1915-1936).

Isolation of latex from A. heterophyllus

The aril, core, sap, tendrils and peduncle of under-ripe jackfruit were used to aseptically collect the latex. The latex was allowed to dry for 12 h at room temperature. Dried latex (2 g) was suspended in 20 mL chloroform and hexane for 5 days. Then the suspended solutions were allowed to stand, and the extract was used to develop hydrophobic paper and oil-resistant paper.

Test for hydrophobicity and oil resistance of latex-coated paper

The latex was isolated by solvent extraction method (Madhavi *et al.*, 2013). Latex extracts of chloroform and hexane were poured on two different rectangular pieces of Whatman No. 1 filter paper. The hydrophobicity was tested by dropping water on latex-coated filter paper using a micropipette and then tested for oil resistance by placing an oil drop on latex-coated filter paper. Both were compared to a filter paper without the latex coating. The water resistance and oil resistance were evaluated by observing the time taken by paper to show hydrophobicity and oil resistance.

Antioxidant activity of A. heterophyllus seeds by DPPH assay

Methanolic extract of sun-dried powdered seeds was used for antioxidant activity. To the extract solution (1.5 mL) of various concentrations 1.5 mL DPPH (1,1-diphenyl-2-picryl-hydrazyl) was added, and reaction mixture incubated for 30 min at 37°C. Absorbance was measured spectrophotometrically at 516 nm. A graph was plotted with antioxidant activity on Y-axis and extract concentration on X-axis. The antioxidant activity (DPPH scavenging activity) was calculated using the following formula (Suma *et al.,* 2021).

$$\text{Antioxidant activity (\%)} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Proximate and mineral analysis of A. heterophyllus seed

For moisture, a well-mixed dried sample (5 g) was taken in a tarred moisture dish and the sample oven-dried at 105±2°C till constant weight. The difference between initial and final weight gave the moisture content in sample (AOAC, 1990). For ash content, 1 g sample in a pre-weighed silica crucible was incinerated by gradually increasing the temperature to 600°C. Then cooled crucible was weighed and ash content calculated (AOAC, 1990). For protein estimation, 0.5 mL sample was taken and 0.1 N sodium hydroxide and 0.5% copper sulphate added, mixed well and incubated. Then Folin's phenol reagent was added and using a spectrophotometer (V-730 Bio Spectrophotometer), the blue colour formed was measured at 660 nm (Lowry *et al.,* 1951). For fat content, 2 mL chloroform and 3 mL methanol were added to 1 g sample. The solution was thoroughly mixed, and kept in a shaker for 1 h. The solution was filtered and leftover residue collected. The residue was mixed with methanol, chloroform and water (in 55:25:12 ratio). The solution was shaken well and allowed to settle. The lower phase was collected and dried in an oven for 3-5 h at 105°C, till constant weight (AOAC, 1990). The carbohydrate content was worked out by subtracting the sum of other proximate components such as protein, fat, moisture and ash from 100 (AOAC, 1990). The mineral content of seeds was performed as per AOAC standard methods (1990).

FTIR characterization of seed powder of A. heterophyllus

Methanolic extracts of sun-dried powdered seeds of *A. heterophyllus* were used for FTIR spectroscopy (Sreeletha *et al.,* 2017). The 2 mg sample was mixed with 100 mg KBr (FT-IR grade) before compressing it to make the sale-disc (3 mm dia.). The disc was placed into the sample holder immediately and FTIR spectra (PerkinElmer Spectrum IR) recorded in the absorption range of 600-4000 cm^{-1}.

Phytochemical analysis of seeds and leaves of A. heterophyllus

The finely powdered seeds and leaves (1 g each) were separately added to 10 mL methanol. The extraction was carried out in a shaker system for 48 h. The seeds/leaf extracts were analysed for tannins, saponins, flavonoids and phenols. For tannin estimation, lead acetate test was followed (Vinod *et al.,* 2010). A few drops of 10% lead acetate were added to the test solution and the formation of precipitate revealed the presence of tannins. For saponins, foam test was followed (Sofowora, 1993). For this, 10 mL distilled water was added to 3 mL aqueous extract solution. The test tube was stoppered, agitated vigorously for 5 min, then kept standing for 30 min, and checked for foam which if stable for 15 sec indicated the presence of saponins. The flavonoids were determined by Shinoda test (Trease and Evans, 2002). For this, 0.5 mL extract was dissolved in ethanol, warmed and filtered. The filtrate was mixed with 3 pieces of magnesium chips along with a few drops of conc. HCl. The development of purple colour indicated the presence of flavonoids. Phenols were determined by Folin-Ciocalteu method (John *et al*., 2014) wherein 0.4 mL Folin-Ciocalteu reagent was added to 1 mL extract, shaken well and left as such for 8 min. Then 4 mL 20% sodium carbonate solution was added and the greenish colour development indicated the presence of phenols.

Antibacterial activity of seeds and leaves of A. heterophyllus by disc diffusion method

The microbial strains for antimicrobial screening were procured from the Institute of Microbial Technology, Chandigarh (India). The antibacterial activity of plant extract was tested against Gram-positive and Gram-negative bacteria by agar disc diffusion method. Gram-positive *Staphylococcus aureus* (MTCC 96) and Gram-negative *Escherichia coli* (MTCC 443) were grown on Soya bean casein digest medium (pH 7.3). Agar media were poured into the plates to a uniform depth of 5 mm and allowed to solidify. The microbial suspensions at 5×10^6 cfu mL^{-1} was streaked over the surface of media using a sterile cotton swab to ensure confluent growth of microbes. The discs (6 mm dia.) used were Whatman No. 1 paper discs. Aliquots of the seed and leaf extracts (10 µL) in chloroform were spotted on filter paper discs. The discs were then aseptically applied to the surface of agar plates at well-spaced intervals. The plates were incubated at 37°C for 24 h and the growth inhibition zones, including disc diameters, were measured. Control discs impregnated with 10 µL o solvent chloroform and streptomycin (2 µg per disc) were used alongside the test discs in each experiment (Tortora *et al*., 2004). A total of 6 extracts from the seeds and leaves were screened for potential antibacterial activity.

The experiments were conducted in a completely randomized block design and each treatment was replicated three times. The data was analyzed by using Origin software (version 7.0383, Origin Lab Corporation, Northampto, USA) and the results expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

The latex of *A. heterophyllus* was isolated by using solvents chloroform and hexane. A whitish cream-coloured solution was obtained. For jackfruit latex extraction, Antony and Tan (2022) used trifluoro-ethanol and aqueous extract, while Wagner and Lackner (2021) used acetone and cyclohexane and Madhavi *et al.* (2013) isolated latex of *A. communis* by using methanol, ethanol and chloroform.

Hydrophobicity and oil-resistance of latex-coated paper

The hydrophobicity (water resistance) and oil resistance were evaluated by observing the time the papers showed hydrophobicity and oil repellence. Tests revealed that the latex of *A. heterophyllus* extracted by using chloroform had a hydrophobicity of 50 min and oil resistance of 1.5 h as compared to the hexane-extracted latex which had a hydrophobicity of 25 min and oil resistance of 1 h. Hydrophobic paper is nowadays getting more attention because of certain properties that allow it to be used as a substitute for plastic packaging. Akash and Nayan (2018) developed hydrophobic paper by using bio-wax layer of *Colocasia esculenta* leaves, while Jyoti *et al.* (2014) used epicuticular wax from the leaves of *Calotropis procera* and *Alstonia scholaris*. Maria *et al.* (2019) used wax from *Colocasia esculenta* leaves and chitin from crab shells to develop hydrophobic paper. Oil-resistant property revealed its potential for food packaging. Zhu *et al.* (2015) developed oil-proof paper from chitosan and chitosan/cationic starch; whereas Juraj *et al*. (2018) improved the oil and grease resistance of paper by surface sizing with aqueous dispersions.

Antioxidant activity of A. heterophyllus seeds

DPPH is a free-radical generating compound and has widely been used to evaluate the free-radical scavenging ability of various antioxidant compounds. Fig. 1 shows the relationship between different jackfruit seed extract concentrations (ppm) and antioxidant activity (%). At 530.6 ppm DPPH scavenging activity was 19.54%, at 1061.33 ppm it was 42.31%, and at 1592 ppm it was 67.42% (Table 1). This signifies that the DPPH scavenging activity increases as the concentration of the extract increases. Burci *et al.* (2015) reported higher antioxidant activity of ethanolic extract of jackfruit seeds and attributed it to the presence of high hydroxyl groups. Sreeletha *et al*. (2017) found

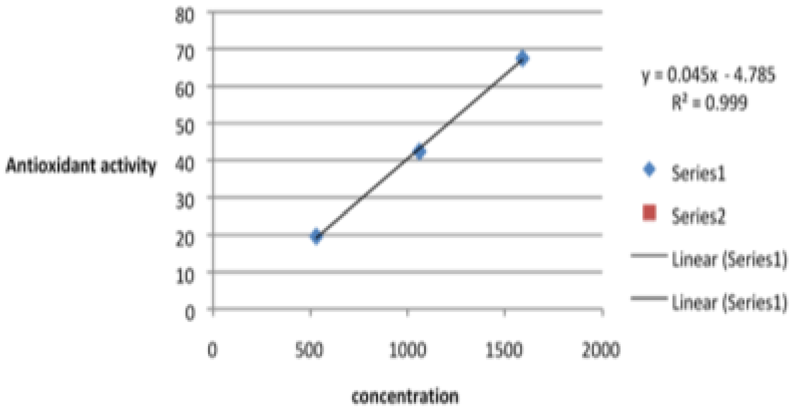

Fig. 1: Antioxidant activity (%) of *Artocarpus heterophyllus* seed at varying concentration (ppm)

IC_{50} values for methanol and ethyl acetate seed extracts of *A. heterophyllus* as 636.5 and 715.86 µg mL^{-1}, respectively.

Proximate and mineral analysis of A. heterophyllus seeds

Proximate analysis of *A. heterophyllus* seeds revealed carbohydrate as major constituent of seed flour(69.59%), followed by protein (10.77%), moisture (11.31%), ash(6.08%) and fat 2.25% (Table 2). Anjali *et al.* (2018) reported almost similar moisture (10%) and ash (6.08%). Contrarily, Albi and Jayamuthunagai (2014) found 2.47% fats in jackfruit. The seeds of *A. heterophyllus* contained sodium, potassium, calcium, magnesium and phosphorus content of 2.4, 12.4, 0.2, 1.1 and 1.5 mg g^{-1}, respectively. Contrarily, Sivaranjini *et al.* (2020) reported higher mineral contents in jackfruit seed flour which may attributed to the geographical and the varietal variations of fruits.

Table 2: Proximate analysis and mineral analysis of *A. heterophyllus* seed

Parameters	Weight (%)
Moisture content	11.31
Ash content	6.08
Protein	10.77
Fat	2.25
Carbohydrate	69.59
Minerals contents	Quantity (mg g^{-1})
- Sodium (Na)	2.40
- Potassium (K)	12.40
- Calcium (Ca)	0.20
- Magnesium (Mg)	1.10
- Phosphorus (P)	1.50

Characterization of A. heterophyllus seeds

The FTIR spectra of methanolic extract *A. heterophyllus* seed showed major peaks at 3318.24, 2856.61, 2831.91, 1239.58 and 1079.09 cm^{-1} (Table 3, Fig. 2). These peak values revealed the presence of certain functional groups like primary alcohol, carboxylic acid, amine salt, amine, aliphatic primary amine, secondary amine, alkyl aryl ether, fluoro compound, alkyne and alkane. Theivasanthi *et al.* (2011) in nano-sized particles of jackfruit seed found the presence of amines, amides, amino acids, carboxylic acids, carbohydrate

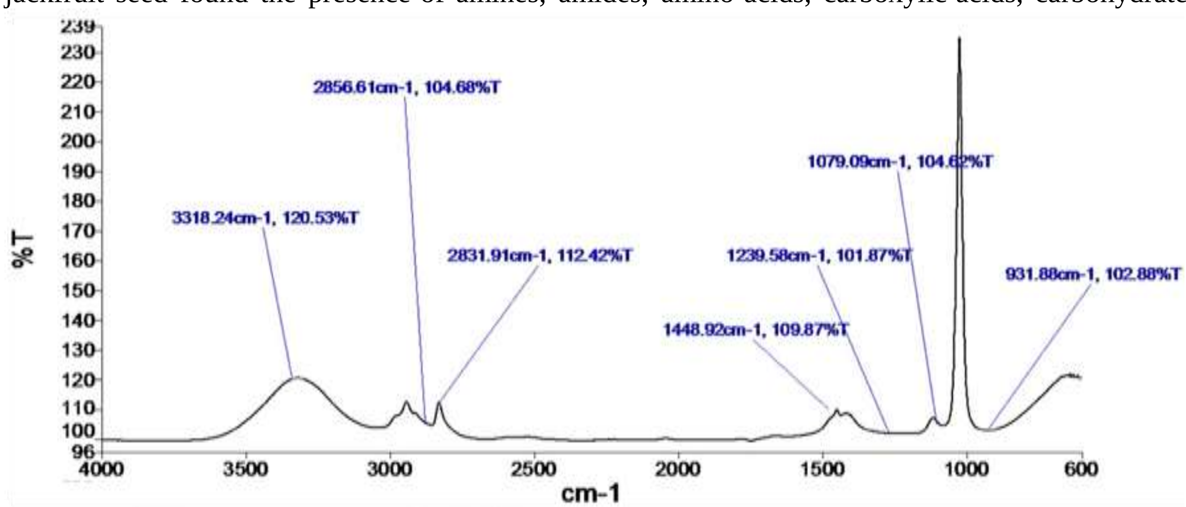

Fig. 2: FTIR spectral analysis of *Artocarpus heterophyllus* seed

Table 3: FTIR analysis of *Artocarpus heterophyllus* seed (Cm^{-1})

Functional group	Absorption Cm^{-1}
Secondary amine: N-H Aliphatic primary amine: N-H Alcohol: O-H Alkyne: C-H	3318.24
Alkane: C-H Amine salt: N-H Alcohol: O-H Carboxylic acid: O-H	2856.61
Amine salt: N-H Alcohol: O-H Carboxylic acid: O-H	2831.91
Amine: C-N Alkyl aryl ether: C-O	1239.58
Primary alcohol: C-O Amine: C-N Fluoro compound: C-F	1079.09

alkanes, alkenes and aromatics while Sreeletha *et al.* (2018) reported the presence of carboxylic acids, ether and C-C linkages.

Phytochemical analysis of seeds and leaves

Phytochemical analysis of methanolic extracts of *A. heterophyllus* seeds and leaves revealed the presence of tannins, saponins (in seeds), flavonoids, and phenols (in leaves) (Table 4). Lengbiye *et al.* (2017) reported that *A. heterophyllus* is a good source of secondary metabolites like flavonoids, terpenoids, steroids, phenols, prenylflavones, glycosides, saponins, alkaloids and tannins. These metabolites have antihelminthic properties. Sreeletha *et al.* (2018) reported that *A. heterophyllus* fruit flour contains carbohydrates, fats, proteins, flavonoids, phenols, carboxylic acids, coumarins, saponins, alkaloids and tannins.

Table 4: Qualitative phytochemical analysis of seeds and leaves of *Artocarpus heterophyllus*

Parameters	Tests	Inference	
		Seeds	Leaves
Tannins	Lead acetate test	+	-
Saponins	Foam test	+	-
Flavonoids	Shinoda's test	-	+
Phenols	Folin-Ciocalteu reagent test	-	+

+ present, - absent

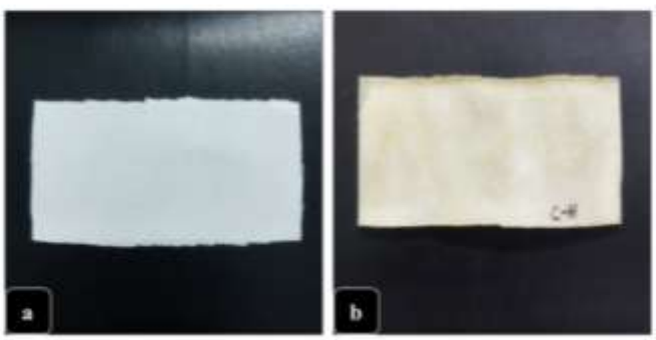

Fig. 4: a) Normal Whatman No. 1 filter paper; b) *Artocarpus heterophyllus* Lam. latex coated Whatman No. 1 filter paper

Antibacterial activity of seeds and leaves

The chloroform extracts of *A. heterophyllus* seeds showed inhibition zone of about 8.2 mm against Gram-positive *Staphylococcus aureus* and 8.8 mm against Gram-negative *Escherichia coli*, while chloroform extract of *A. heterophyllus* leaves did not show any zone of inhibition against these test bacterial pathogens (Table 5). Sreeletha *et al. (*2017) found highest antibacterial activity of ethyl acetate and acetone extract jackfruit seeds against *S. aureus* (20 mm) and *K. pneumonia* (16 mm), whereas hexane extract showed no such activity against these pathogens.The aqueous extract did not show any activity against test pathogens. Antony *et al.* (2022) reported that trifluoroethanol latex extract of *A. heterophyllus* possessed antibacterial activity against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus* sp.

Conclusion: *Artocarpus heterophyllus* Lam. is rich in nutritional and mineral value, along with strong antioxidant and antibacterial properties. The low-fat content of the seeds makes them a good constituent of fat-free diet. The results of the study emphasise the importance of consuming cooked seeds and boosting the production of value-added food products. The presence of functional groups in seeds helps in development of pharmaceutically active drugs, and some important phytochemicals in both seeds and leaves make the study more prominent. The study reveals

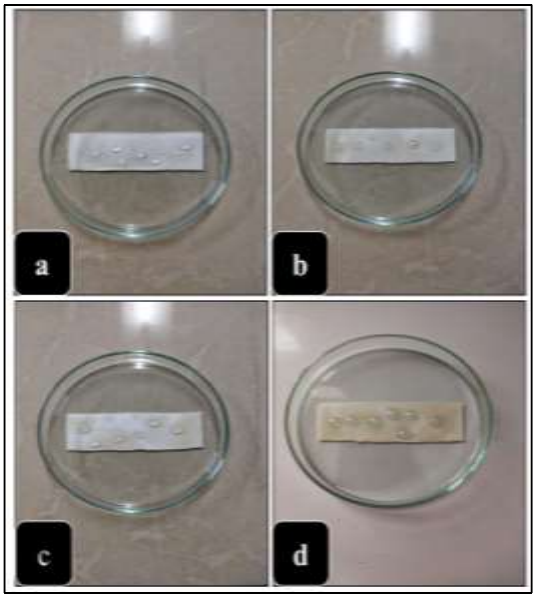

Fig. 5: Chloroform latex extract coated paper showing a) hydrophobicity b) oil resistance; and hexane latex extract coated paper showing c) hydrophobicity d) oil resistance

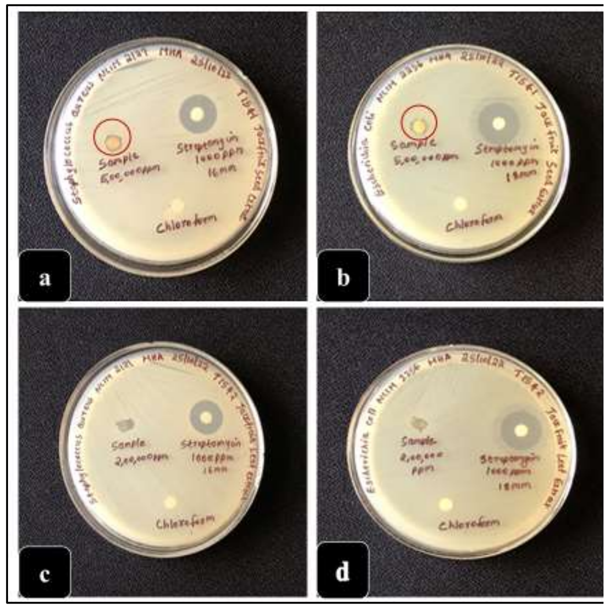

Fig. 6: Antibacterial activity of *Artocarpus heterophyllus* seed (a. *Staphylococcus aureus* b. *Escherichia coli*), and leaves (c. *Staphylococcus aureus* d. *Escherichia coli*)

Table 5: Antibacterial screening of chloroform extracts of seeds and leaves of *Artocarpus heterophyllus*

Microorganisms	Diameter of inhibition zone including disc (mm) (mean ± standard deviation)			
	Seeds	Leaves	Streptomycin (+ve control)	Chloroform (-ve control)
Staphylococcus aureus (MTCC96)	8.2 ± 0.12	6.02 ± 0.03	22 ± 1.30	6.3 ± 0.33
Escherichia coli (MTCC 443)	8.8 ± 0.16	6.00 ± 0.00	24 ± 0.80	6.5 ± 0.11

the potential of seeds, which help in value addition and can be consumed as a good source of nutritional, mineral and antioxidant components. The latex can be used to develop hydrophobic and oil resistant paper which can used for food packaging. The study underlines the importance of *A. heterophyllus* for development of new innovative eco-friendly products, which can be used in several ways in our day-to-day life.

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